Cell Metabolism
Meeting Report

Look Who's Talking: Nuclear Receptors in the Liver and Gastrointestinal Tract

Michael Downes[1,*] and Christopher Liddle[2]
[1]Howard Hughes Medical Institute, Gene Expression Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, USA
[2]Storr Liver Unit, Westmead Millennium Institute, University of Sydney, Westmead Hospital, Westmead, NSW 2145, Australia
*Correspondence: downes@salk.edu
DOI 10.1016/j.cmet.2008.02.006

The Nuclear Receptors in Liver and Digestive Diseases research workshop was held in Rockville, Maryland, on November 7 and 8, 2007, under the auspices of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. Over 130 researchers from around the world gathered to assess the pathophysiology of nuclear receptors in the liver and gastrointestinal tract and to explore their potential use as therapeutic targets. This review covers some of the highlights of the meeting, including important areas of future investigation.

Introduction

Knowledge of the physiology and pathophysiology of nuclear receptors (NRs) in the gut and liver is expanding rapidly, and this NIDDK-sponsored research workshop provided the opportunity to take stock of what we have learned and to identify priorities for future research and clinical intervention. The meeting was opened by a presentation by Ron Evans (Salk Institute) on the recently completed spatial and temporal expression profiles for the 49 murine NRs (Bookout et al., 2006; Yang et al., 2006). Of interest to this meeting, it was found that a large number of NRs are expressed in the liver and gut, 37 and 41 respectively. The specific NRs and their association with liver and gut disorders discussed at this meeting are highlighted in Figure 1. Eight NRs are effectively limited in expression to the liver and gut, including the farnesoid X receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), and hepatocyte nuclear factor 4α (HNF4α). Acting as intracellular sensors for lipophilic molecules, these restricted NRs have essential roles in a diversity of cellular processes including digestion, lipid and energy homeostasis, and inflammation. Extensions of the NR expression profiling studies are now underway to explore subpatterns of NR expression within minority cell populations of larger tissues. Preliminary results have revealed discrete expression patterns in specific cell types. For example, the hepatic stellate cell (HSC), which represents only a small percentage of the liver mass, plays a pivotal role in hepatic fibrogenesis and vitamin A storage, as reviewed in a presentation by Scott Friedman (Mount Sinai School of Medicine). Profiling of HSCs reveals a very different NR complement as compared to hepatocytes, the dominant hepatic cell type. These types of observations are important not only for the purposes of understanding these cells' molecular and cellular contributions to normal and disease states but also in identifying differential targets for pharmacological manipulation. Rodent and human tissue and cell profiles can be found in the Nuclear Receptor Signaling Atlas (NURSA; http://www.nursa.org), an NIH-funded data repository designed to accrue, coordinate, and communicate experimental data and related information pertinent to NRs and their functions (Margolis et al., 2005).

The meeting continued with over 25 presentations of both recently published and as yet unpublished research accompanied by lively discussion of how recent advances in the understanding of the function of specific NRs in liver and gastrointestinal tract could be translated into the clinical setting. Below are some highlights.

Bile Acids, Oxysterols, Fibroblast Growth Factors, and the Enterohepatic Axis

Although bile acids are metabolites of cholesterol that facilitate absorption of dietary fats, it is now apparent that a subset of bile acids act as enterohepatic hormones that coordinate several aspects of digestion and energy homeostasis. Like their classical steroid hormone relatives, certain bile acids are ligands for NRs, specifically FXR, PXR, and the vitamin D receptor (VDR) (reviewed in Zollner et al., 2006), while related oxidized cholesterol metabolites (oxysterols) are ligands for the liver X receptors (LXRs). In addition, it is now apparent that NR regulation of fibroblast growth factors (FGFs) is important for their ability to maintain metabolic homoeostasis of the body.

Fasting versus Fed: NR and FGF Signaling between Gut and Liver

In two thought-provoking presentations from the Mangelsdorf/Kliewer laboratory (University of Texas Southwestern Medical Center), the regulatory roles of NRs in metabolic homeostasis during the nutritional extremes of the fasted and fed states were covered. These studies showed that in the fasted and fed states, peroxisome proliferator-activated receptor α (PPARα) and FXR respectively induce the expression of a specific class of autocrine-functioning FGFs, thereby identifying new potential therapeutic targets. In the early fed state, oxysterols in the liver, rapidly formed by as yet unknown mechanisms coupled to dietary cholesterol load, activate LXR to engage gene programs that promote the conversions of dietary fats and carbohydrates to triglycerides as well as promoting the elimination of excess cholesterol through metabolism to bile acids (Kalaany and Mangelsdorf, 2006). In the late fed state, bile acids that have been excreted from the liver bind to FXR in the distal small intestinal mucosa to promote intestinal bile acid recycling and induce expression of FGF15 (FGF19 in humans) in intestinal enterocytes. FGF15 is carried in the portal circulation to the liver, where along with bile acid-activated hepatic FXR it decreases bile acid

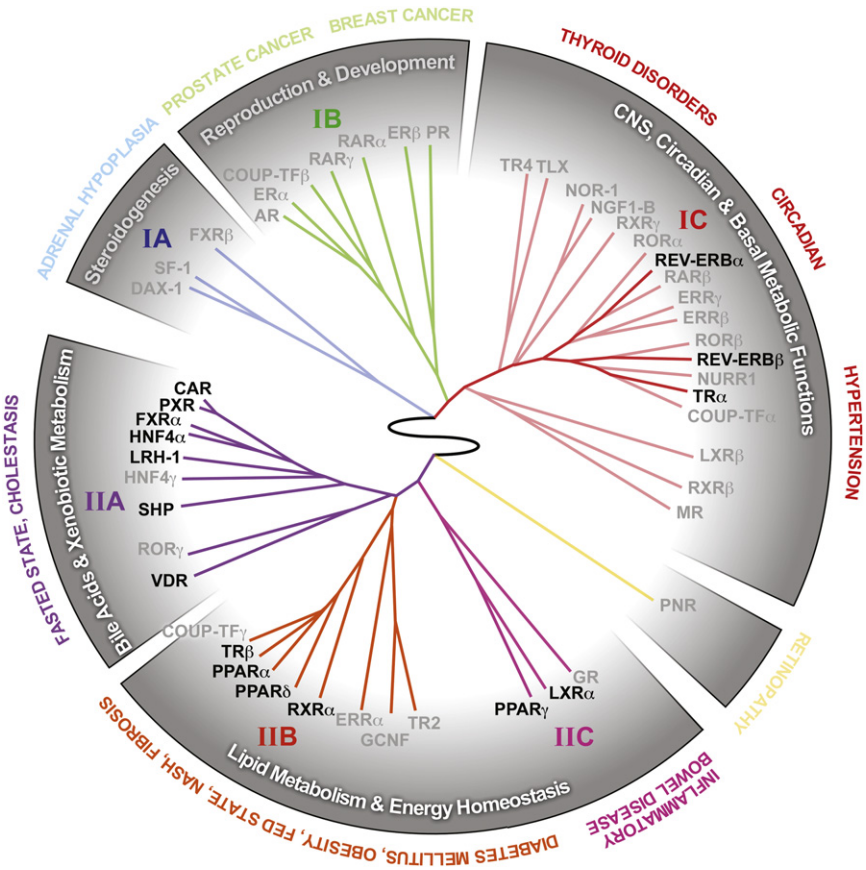

Figure 1. Nuclear Receptors in Liver and Gut Pathophysiology

The nuclear receptors and their associated liver and gut diseases discussed at the NIDDK Nuclear Receptors in Liver and Digestive Diseases research workshop (Rockville, Maryland, November 2007) are highlighted in this dendrogram showing the relationship between receptor expression, function, physiology, and disease. (Modified from Bookout et al., 2006.)

synthesis through repression of CYP7A1 (Inagaki et al., 2005). In addition, FGF15 causes relaxation of the gallbladder, allowing it to refill with bile (Choi et al., 2006). Bile acid-activated FXR in the liver induces expression of genes that increase gluconeogenesis and promote triglyceride clearance and fatty acid β-oxidation concomitantly with a reduction of lipogenic gene transcription, effects largely opposed to those of LXR (Kalaany and Mangelsdorf, 2006). In the fasted state, free fatty acids are sensed by PPARα, which in turn promotes utilization of stored fat in adipose tissue and promotes hepatic ketogenesis to provide an alternate energy supply to glucose for tissues such as the brain, heart, and muscle. PPARα also induces hepatic expression of FGF21, a "hepatokine" that contributes locally to ketogenesis and communicates between the liver and adipose tissue, promoting fat mobilization through lipolysis in adipocytes. FGF21 also induces an energy-conserving hibernation-like state, torpor, manifested by a decrease in body temperature (Inagaki et al., 2007).

These presentations describing mechanisms of normal nutrient and energy homeostasis were an appropriate precursor to presentations covering the "new" diseases of the late twentieth and twenty-first centuries that have their basis in excessive nutrient intake, as covered in the following section.

NRs, NASH, and the Metabolic Syndrome

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in affluent societies, representing the hepatic metabolic consequence of relative overnutrition and altered diet composition in the setting of reduced physical activity and increased sedentary behavior. The sometimes progressive, inflammatory form termed nonalcoholic steatohepatitis (NASH) may lead to the development of hepatic fibrosis and cirrhosis. Insulin resistance is almost universal in NAFLD, as is the presence of visceral obesity (reviewed in George and Liddle, 2008). The consensus of the meeting was that NRs are viable therapeutic targets in the treatment of these hepatic disorders. To date, human studies of NR ligands in the pharmacotherapy of NASH have concentrated on PPARγ agonists such as rosiglitazone and pioglitazone. While these agents improve insulin sensitivity and decrease hepatic steatosis, their clinical utility is limited by weight gain. Considerable effort is being directed toward identifying additional NR-targeted ligands of therapeutic value, as demonstrated by Joel Levine's (University of California, San Diego) study of NR expression in human NASH liver biopsies.

PPARδ is currently the most promising NR target for NASH and the metabolic syndrome due to its powerful regulatory actions on fat, skeletal muscle, liver, and the heart. Its activation by fatty acids enhances fatty acid transport and oxidation, improves glucose homeostasis via inhibition of hepatic glucose output, reduces macrophage inflammatory responses, and dramatically increases circulating high-density lipoprotein levels (reviewed in Barish et al., 2006). Richard Heyman (Kalypsys, Inc.) reported that in mice fed a high-fat diet (58% fat), treatment with the selective PPARδ ligand K3010 causes a reduction in visceral fat and total body fat and a reduction in both hepatic steatosis and inflammation. Discussions of these findings revolved around whether these improvements in NASH reflect direct hepatic effects or secondary effects via enhanced oxidative metabolism in skeletal muscle and adipose tissue, which increases glucose utilization, Glut4 expression, and processing of long-chain fatty acids in adipose tissue. The recently reported phase I human study of the PPARδ agonist GW501516 (GlaxoSmithKline) in moderately obese males supports the Kalypsys data showing increased fatty acid metabolism in skeletal muscle and reduction in hepatic fat (Riserus et al., 2008). Longer-term studies of patients with insulin resistance, the broader metabolic syndrome, and NASH are eagerly anticipated.

NRs and Cholestatic Liver Diseases

Cholestatic liver diseases are characterized by impaired bile flow and accumulation of toxic bile constituents such as bile acids in the liver, with resultant liver injury and fibrosis. Pharmacological therapy for chronic cholestatic disorders is limited, as reviewed by Allan Hoffman (University of California, San Diego) and Michael Trauner (Medical University of Graz, Austria). Ursodeoxycholic acid is the only cholestatic disease-modifying therapy with evidence of efficacy, so new therapeutic approaches are essential. Recent studies are beginning to identify NR targets for the management of cholestasis. These studies have focused largely on FXR, which represents an interesting dichotomy, as both agonists and antagonists may have beneficial effects for cholestatic liver disease. When the biliary tract is partially blocked, activation of FXR results in enhanced bile flow through increased hepatocyte apical bile acid transporter (ABCB11) expression, augmenting excretion of bile acids into the biliary canaliculus and thereby reducing bile acid accumulation in the liver. However, in complete biliary obstruction this approach is detrimental, resulting in hydrostatic bile infarcts due to rupture of the canals of Herring. Thus, in bile duct-ligated mice, FXR knockouts fare better than wild-type, and upregulation of the basolateral bile acid export pump Mrp4 (Abcc4) facilitates export of bile acids out of the hepatocyte and into the circulation for renal excretion (Marschall et al., 2006; Stedman et al., 2006). These findings led to the proposal of two potential therapies for cholestatic diseases: FXR agonists may be the drug of choice for small duct biliary diseases, while FXR antagonists may be better suited when large duct obstruction is present.

Efforts to improve the efficacy of ursodeoxycholic acid have led to the identification of 24-norursodeoxycholic acid, administration of which in human subjects induces a bicarbonate-rich choluresis (increased bile flow) and reduces the cholesterol and phospholipid content of bile. Animal studies presented at the meeting suggest that 24-norursodeoxycholic acid may be superior for the treatment of cholestasis, and unpublished data suggest that its favorable actions may in part be mediated through activation of CAR, a NR capable of activating multiple phase 1 and 2 detoxification enzymes in the liver as well as modulating several hepatocyte transporters (reviewed in Handschin and Meyer, 2005).

Inflammatory Bowel Disease

In contrast to the liver, much less is known about the roles of NRs in health and disease in the gut. Inflammatory bowel disease (IBD) is a major clinical problem that can be refractory to even the most potent anti-inflammatory and immunosuppressive therapies. While the etiology of this disease remains unknown, PPARγ is highly expressed in the colonic epithelium, and animal studies have shown that PPARγ ligands are effective in reducing inflammation in chemically induced models of colitis such as dextran sodium sulfate (DSS)-treated mice. Potential mechanisms of how PPARγ exerts ligand-dependent transrepression of Toll-like receptor target genes were proposed by Chris Glass (University of California, San Diego) (Straus and Glass, 2007). Gary Wu (University of Pennsylvania) presented exciting clinical findings from a soon to be published study of treatment of patients with mild to moderately active ulcerative colitis with the PPARγ agonist rosiglitazone (52 patients 4 mg twice daily versus 53 patients placebo). A reduction in disease activity index was observed in 44% of patients receiving rosiglitazone compared to 23% of controls, and clinical improvement was usually observed within 4 weeks of commencing therapy (Lewis et al., 2008). Further studies are needed to determine the place of PPARγ agonists in the management of IBD, but their relative lack of toxicity makes them an attractive treatment option compared to existing therapies. The success of this small study raises the possibility that these drugs may be useful prophylactically to prevent disease flares.

Other NRs may also play protective roles in intestinal and colonic mucosa. PPARδ knockout mice are more susceptible to DSS-induced colitis (Hollingshead et al., 2007), and a potent PXR agonist ameliorates colonic inflammation in this model (Shah et al., 2007). Taken together, these early clinical and preclinical findings suggest plenty of scope for NR agonists in luminal gastrointestinal diseases, particularly those where inflammation is a predominant feature of pathogenesis, and further clinical studies should now be a research priority given that drugs that target these receptors are already available or soon will be.

REV-ERBα: An Orphan NR Finds a New Home?

The importance of the circadian clock in regulating metabolic homeostasis is becoming increasingly apparent. The NRs REV-ERBα and retinoic acid receptor-related orphan receptor α (RORα) are integral players in circadian rhythms through their direct regulation of the cyclic expression of brain and muscle Arnt-like protein 1 (BMAL1), a key component of the clock. REV-ERBα is transcribed in a cyclic manner throughout the body and had been thought of as a constitutive repressor, as its ligand-binding domain (LBD) lacks the domain required for coactivator recognition. Evidence was presented at the meeting from both the Lazar (University of Pennsylvania) and Burris (Pennington Biomedical Research Center) groups (Raghuram et al., 2007; Yin et al., 2007) that the mammalian REV-ERBα, like its fly homolog (Reinking et al., 2005), also associates with heme. However, in contrast to the fly, heme may bind in mammals in a reversible manner influencing the receptor's ability to repress transcription of hepatic metabolic genes. In vivo, heme stabilizes the interaction of REV-ERBα with the nuclear corepressor (NCoR) complex, facilitating enhanced repression of target genes. This finding, combined with the circadian cycling of intracellular heme levels, led to the exciting proposal that REV-ERBα is a heme sensor and may provide the molecular link to how circadian rhythms influence metabolic homeostasis. However, the mechanism of how heme stabilizes the interaction of NCoR with REV-ERBα remains elusive. In contrast to the in vivo data, addition of heme results in the dissociation of corepressor CoRNR box peptides from the REV-ERBα ligand-binding domain in well-characterized in vitro assays. In addition, it is unclear what the structural consequences are of the H602F mutation in the REV-ERBα LBD used in these studies to demonstrate that loss of heme binding leads to loss of NCoR binding. Previous detailed structural modeling indicated that this amino acid makes important van der Waals interactions with other amino acids within the LBD to stabilize the positioning of helix 11 and helix 3 (Renaud et al., 2000). Detailed cocrystal structures of heme bound to the REV-ERBα receptor are eagerly awaited. Collectively, these findings raise the questions of whether REV-ERBα should now

be moved into the class of receptors that have adopted ligands and whether it can be pharmacologically manipulated so as to allow detailed mechanistic studies of the link between circadian rhythm and hepatic metabolism.

Future Directions

Many unanswered questions concerning the distribution and function of NRs and associated coactivator and corepressor factors in the liver and gastrointestinal tract remain. As mentioned in the introduction of this review, we need a better understanding of NR expression in the individual cell types that comprise tissues, including cells that have predominantly metabolic functions, those that have central roles in injury and inflammation, and particularly stem cell populations given their increasingly recognized role in disease and tissue repair. For example, in the liver we are finding that nonparenchymal cells have very different NR expression patterns compared to hepatocytes. The unexpected expression of some "nonhepatic" NRs suggests entirely new functions for these cell types and highlights the possibility of novel therapeutics. Furthermore, again taking the liver as an example, the humble hepatocyte exhibits zonal functional specialization within the hepatic lobular architecture, yet we know nothing about the cause-and-effect relationships between NRs and hepatocyte phenotypes.

The next challenge for the NR field in the area of liver and gastrointestinal disease is to push the translational boundary closer to the bedside by performing more detailed studies of NR distribution and function in human tissues. Much has been learned using animal and cell-based models, but effective translational research could be rapidly advanced through detailed knowledge of NR expression patterns in both normal and diseased human tissues. In relation to IBD and cholestatic liver diseases, where we are already at or near the bedside, there is plenty of scope for medicinal chemists to develop new or improved NR ligands to facilitate clinical studies, including FXR agonists and antagonists for cholestasis as well as PPARγ ligands designed to target the intestinal mucosa in IBD.

Experience gained using the thiazolidinedione class of PPARγ agonists for the treatment of type 2 diabetes suggests that targeting single NRs may not be the most effective strategy, just as in vivo fatty acids activate all three PPARs. Selection of drug targets requires a thorough understanding of NR function across multiple tissues, including the impact of modulation of several NRs at once. This approach is presently undergoing late-phase clinical trials with drugs that target two or all three members of the PPAR family.

In the context of the liver and gastrointestinal tract, a major focus of NR research is in metabolism and homeostasis. It therefore follows that increased use of powerful metabolomic tools should expand our understanding of NR function, particularly when these tools are applied to genetically modified animals or, ideally, to the human situation in both health and disease, a proposition that was deftly argued by Frank Gonzalez (National Cancer Institute, NIH) in his presentation (reviewed in Idle and Gonzalez, 2007). Some of the more obvious targets for research within the scope of this workshop include bile acids, oxysterols, fatty acids, and metabolic disturbances associated with obesity and insulin resistance.

Finally, it is worth considering that bile acids and possibly oxysterols may have far broader functions than have been traditionally ascribed to them. They are enterohepatic hormones that coordinate digestion, energy storage, and very likely energy expenditure. It has been previously recommended that bile acids be collectively referred to as "cholanoids" (Hofmann et al., 1992), a term that may better reflect their pivotal role as signaling molecules.

ACKNOWLEDGMENTS

We are indebted to the speakers for allowing us to cover their work in this review. We apologize to those participants whose presentations we were unable to cover due to space restrictions.

REFERENCES

Barish, G.D., Narkar, V.A., and Evans, R.M. (2006). J. Clin. Invest. *116*, 590–597.

Bookout, A.L., Jeong, Y., Downes, M., Yu, R.T., Evans, R.M., and Mangelsdorf, D.J. (2006). Cell *126*, 789–799.

Choi, M., Moschetta, A., Bookout, A.L., Peng, L., Umetani, M., Holmstrom, S.R., Suino-Powell, K., Xu, H.E., Richardson, J.A., Gerard, R.D., et al. (2006). Nat. Med. *12*, 1253–1255.

George, J., and Liddle, C. (2008). Mol. Pharm. *5*, 49–59. Published online December 12, 2007. 10.1021/mp700110z.

Handschin, C., and Meyer, U.A. (2005). Arch. Biochem. Biophys. *433*, 387–396.

Hofmann, A.F., Sjovall, J., Kurz, G., Radominska, A., Schteingart, C.D., Tint, G.S., Vlahcevic, Z.R., and Setchell, K.D. (1992). J. Lipid Res. *33*, 599–604.

Hollingshead, H.E., Morimura, K., Adachi, M., Kennett, M.J., Billin, A.N., Willson, T.M., Gonzalez, F.J., and Peters, J.M. (2007). Dig. Dis. Sci. *52*, 2912–2919.

Idle, J.R., and Gonzalez, F.J. (2007). Cell Metab. *6*, 348–351.

Inagaki, T., Choi, M., Moschetta, A., Peng, L., Cummins, C.L., McDonald, J.G., Luo, G., Jones, S.A., Goodwin, B., Richardson, J.A., et al. (2005). Cell Metab. *2*, 217–225.

Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswara, V., Li, Y., Goetz, R., Mohammadi, M., Esser, V., et al. (2007). Cell Metab. *5*, 415–425.

Kalaany, N.Y., and Mangelsdorf, D.J. (2006). Annu. Rev. Physiol. *68*, 159–191.

Lewis, J.D., Lichtenstein, G.R., Deren, J.J., Sands, B.E., Hanauer, S.B., Katz, J.A., Lashner, B., Present, D.H., Chuai, S., Ellenberg, J.H., et al. (2008). Gastroenterology. Published online December 7, 2007. 10.1053/j.gastro.2007.12.012.

Margolis, R.N., Evans, R.M., O'Malley, B.W., and NURSA Atlas Consortium (2005). Mol. Endocrinol. *19*, 2433–2436.

Marschall, H.U., Wagner, M., Bodin, K., Zollner, G., Fickert, P., Gumhold, J., Silbert, D., Fuchsbichler, A., Sjovall, J., and Trauner, M. (2006). J. Lipid Res. *47*, 582–592.

Raghuram, S., Stayrook, K.R., Huang, P., Rogers, P.M., Nosie, A.K., McClure, D.B., Burris, L.L., Khorasanizadeh, S., Burris, T.P., and Rastinejad, F. (2007). Nat. Struct. Mol. Biol. *14*, 1207–1213.

Reinking, J., Lam, M.M., Pardee, K., Sampson, H.M., Liu, S., Yang, P., Williams, S., White, W., Lajoie, G., Edwards, A., and Krause, H.M. (2005). Cell *122*, 195–207.

Renaud, J.P., Harris, J.M., Downes, M., Burke, L.J., and Muscat, G.E. (2000). Mol. Endocrinol. *14*, 700–717.

Riserus, U., Sprecher, D., Johnson, T., Olson, E., Hirschberg, S., Liu, A., Fang, Z., Hegde, P., Richards, D., Sarov-Blat, L., et al. (2008). Diabetes *57*, 332–339. Published online November 16, 2007. 10.2337/db07–1318.

Shah, Y.M., Ma, X., Morimura, K., Kim, I., and Gonzalez, F.J. (2007). Am. J. Physiol. Gastrointest. Liver Physiol. *292*, G1114–G1122.

Stedman, C., Liddle, C., Coulter, S., Sonoda, J., Alvarez, J.G., Evans, R.M., and Downes, M. (2006). Proc. Natl. Acad. Sci. USA *103*, 11323–11328.

Straus, D.S., and Glass, C.K. (2007). Trends Immunol. *28*, 551–558.

Yang, X., Downes, M., Yu, R.T., Bookout, A.L., He, W., Straume, M., Mangelsdorf, D.J., and Evans, R.M. (2006). Cell *126*, 801–810.

Yin, L., Wu, N., Curtin, J.C., Qatanani, M., Szwergold, N.R., Reid, R.A., Waitt, G.M., Parks, D.J., Pearce, K.H., Wisely, G.B., and Lazar, M.A. (2007). Science *318*, 1786–1789.

Zollner, G., Marschall, H.U., Wagner, M., and Trauner, M. (2006). Mol. Pharm. *3*, 231–251.